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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/687,246	10/12/2000	William G. Nelson	JHU1660-1	1350

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EXAMINER
SOUAYA, JEHANNE E

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 01/10/2003 21

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/687,246	Nelson et al
Examiner	**Art Unit**
Jehanne Souaya	1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on Oct 29, 2002 .

2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 1-51 and 76-85 is/are pending in the application.

4a) Of the above, claim(s) 76-83 is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 1-51, 84, and 85 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on Oct 12, 2000 is/are a) ☒ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4) ☒ Interview Summary (PTO-413) Paper No(s). 21
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Currently claims 1-51, 84, and 85 are under consideration. Claims 76-83 have been withdrawn from consideration as being drawn to non elected inventions. All the amendments and arguments have been thoroughly reviewed but are deemed insufficient to place this application in condition for allowance. Any rejections not reiterated are hereby withdrawn. The following rejections are either newly applied or are reiterated. They constitute the complete set being presently applied to the instant Application. Response to Applicant's arguments follow. This action is made FINAL.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

3. The rejection under 35 USC 112/2nd paragraph with regard to claims 1, 2, and 40 (section 7B) and the recitation of "glutathione S transferase nucleic acid" or "GST nucleic acid" and claims 16 and 28 (section 7J) with regard to the recitation of "GST DNA" or "GST RNA" is withdrawn in view of applicants arguments that such encompasses any GST nucleic acid. From the teachings in the specification, (for example, p. 1, lines 27-28; p. 2, lines 7-8; and p. 6, lines 12-14) it is clear that terms such as "glutathione S transferase nucleic acid", "GST nucleic acid", "GST DNA", and "GST RNA" refer to nucleic acid encoding glutathione S transferase.

The rejection under 35 USC 112/2nd paragraph in sections 7A and 7E of the previous office action is withdrawn in view of applicants arguments.

The rejection under 35 USC 112/2nd paragraph in sections 7C, 7D, 7F, 7G, 7I, 7K, and 7L of the previous office action are withdrawn in view of the amendments to the claims.

Maintained Rejections

Claim Rejections - 35 USC § 112

Indefinite

4. Claims 14 and 15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 is indefinite as it is unclear where in the method steps of claim 6, the method step of claim 14 is carried out.

Response to Arguments

The response asserts that the examiner's position that it is unclear as to where in the method of claim 6 the step of claim 14 is carried out is irrelevant because it is well established that there is no chronological order implied in the steps of a method claim, and that claim 14 simply recites an additional step. This argument has been thoroughly reviewed but found unpersuasive because claim 14 recites "contacting the nucleic acid with a methylation sensitive restriction endonuclease" whereas claim 6 contains more than one recitation of a "nucleic acid",

therefore it is not clear that claim 14 is an additional step, that is to be performed during or after the 'detecting' step of claim 6. If this is the intention of claim 14, this rejection could be easily overcome by reciting instead "contacting the methylated CpG containing GST nucleic acid with...".

Enablement

5. Claims 1-51, 84 and 85 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting hepatocellular carcinoma (HCC) or liver cancer in humans comprising obtaining a specimen that contains a nucleic acid encoding GSTP1, wherein the specimen is selected from the group consisting of hepatic tissue, bile, and blood, and detecting a hypermethylated CpG promoter region at -539 to -239 from the transcription start site of the nucleic acid encoding GSTP1, wherein a hypermethylated CpG promoter region at -539 to -239 from the transcription start site of the nucleic acid encoding GSTP1 in the specimen as compared to the level of methylation of the promoter region at -529 to -239 from the transcription start site of the nucleic acid encoding GSTP1 in normal hepatic tissue, is indicative of hepatocellular carcinoma or liver cancer in humans; does not reasonably provide enablement for a method for detecting a hepatic cell proliferative disorder comprising detecting a methylated CpG containing GST in a biological fluid or detecting a decrease in the level of GST RNA as compared to GST RNA levels in normal cells. The specification does not

enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

There are many factors to be considered when determining whether there is sufficient evidence to support determination that a disclosure does not satisfy the enablement requirements and whether any necessary experimentation is undue (See *In re Wands,* 858 F. 2d 731, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). These factors include, but are not limited to:

Quantity of Experimentation Necessary
Amount of Direction and Guidance
Presence and Absence of Working Examples
Nature of the Invention
Level of predictability and unpredictability in the art

The claims are broadly drawn to detecting any hepatic cellular proliferative disorder in a human or any subject, by detecting methylation of any region of any GST nucleic acid or by detecting decreased levels of any GST RNA in any biological fluid. The claims are further limited to detecting hypermethylation of GST DNA in any region as well as a promoter, and more specifically to the region -539 to -239 from the transcriptional start site. The claims are also further limited to a π family GST and more specifically to GSTP1. The claims are also limited to detecting methylation of any GST nucleic acid and hepatitis B or C virus and correlating such to any hepatic cellular proliferative disorder. The specification, however, does not enable the skilled artisan to make or use the invention commensurate in scope with the claims. Although some claims are of a more limited scope, they are still too broad such that the specification does not enable the broad scope of the claims.

The specification teaches that the invention is based on the observation that human liver carcinogenesis proceeds via accumulation of CpG island hypermethylation changes at GSTP1 and that such hypermethylation was detected in 85% of HCC cases studied. The specification (p. 36) teaches that Hep3B HCC cells tested, failed to express either GSTP1 polypeptides or GSTP1 mRNA (para 1). The specification further teaches that GSTP1 promoter alleles present in Hep3B HCC cells manifested abnormal hypermethylation. The specification teaches that when Hep3B HCC cells were exposed to 5-aza-dC, an inhibitor of DNA methyltransferases, GSTP1 mRNA expression increased (para 2). The specification, at page 38, teaches that 19 of 20 HCC cases appeared devoid of GSTP1 polypeptide expression (table 1) and that DNA from 17 of the 20 HCC specimens showed somatic hypermethylation changes in at least 1 GSTP1 allele (p. 40. Ines 12-13). The specification further teaches that 10 of 20 HCC cases had detectable hepatitis B virus DNA (HBV) among genomic DNA from HCC tissue or from adjacent tissue (p. 41) and that abnormal GSTP1 promoter DNA hypermethylation was present in HCC DNA in 7 out of 10 cases in which HBV was present and in 10 out of 10 cases in which HBV DNA was not detected.

The claims are broadly drawn to detecting any hepatic cell proliferative disorder, however the only proliferative disorder analyzed by the specification is hepatocellular carcinoma. The specification provides no guidance or working examples that any hepatic cell proliferative disorder, such as a cyst, can be detected by detecting any methylated CpG containing nucleic acid encoding GST, or any hypermethylated CpG containing nucleic acid encoding GSTP1, or a decrease in the level of GST RNA. With regard to detecting a methylated CpG containing GST

nucleic acid in any biological fluid, the specification provides no guidance or working examples that hepatocellular carcinoma can be detected by detecting hypermethylated GST nucleic acid or decreased levels of GST mRNA in a urine sample or ejaculate, for example. Given that the specification teaches that the hypermethylation of the GSTP1 promoter region analyzed appeared to be somatic (p. 40, lines 3-4), the specification provides no teaching or guidance that cancerous hepatocytes could be predictably and reproducibly detected in a urine or ejaculate specimen. To practice the invention as broadly as it is claimed, the skilled artisan would have to screen biological fluid samples from a large number of patients with any hepatic cell proliferative disorder, as well as obtaining biological fluid samples from healthy controls to determine whether hypermethylated CpG containing GST or decreased levels of GST mRNA could be detected in any biological fluid sample, and further, if any hepatic cell proliferative disorder could be diagnosed from such samples. Such recitation in the claims provide the skilled artisan with an invitation to experiment. Such experimentation is considered undue, however, as it requires trial and error analysis, the results of which are unpredictable.

With regard to detecting an association between methylated CpG containing GST nucleic acid and any hepatic cell proliferative disorder, the specification provides no guidance or working examples that any hepatic cell proliferative disorder can be detected by only detecting methylated CpG containing GST. Further, Herman et al (US Patent 6,017,704) teaches that human genes are known to be methylated (see col. 4, lines 1-5), including GST pi (human GST pi is GSTP1), and that hypermethylation of GST pi is associated with neoplastic tissue.

Therefore, because the art teaches that GSTP1 inherently exists in a methylated state, the skilled artisan would not be able to detect a hepatic cell proliferative disorder based solely on the detection of methylated CpG containing GST nucleic acid. Furthermore, although the specification teaches that DNA hypermethylation was present in HCC DNA in 7 out of 10 cases in which HBV was also detected, the specification does not teach the detection of HCV. Furthermore, the specification does not provide any guidance or working examples that detecting HBV or HCV and detecting methylated CpG containing GST nucleic acid is indicative of a hepatic cell proliferative disorder. Since CpG containing GSTP1 nucleic acid is inherently methylated, the detection of HBV along with methylated GSTP1 could be indicative of hepatitis B infection, but does not necessarily indicate to one of skill in the art that a hepatic cell proliferative disorder, such as liver cancer or a cyst, is also present.

With regard to detecting a hepatic cell proliferative disorder in any subject (for example, claims 28-51, 84, and 85), the specification provides no working examples that a hepatic cell proliferative disorder in any organism or mammal, such as a rat for example, can be detected by detecting any methylated CpG containing nucleic acid encoding GST, or any hypermethylated CpG containing nucleic acid encoding GSTP1, or a decrease in the level of GST RNA. The art is unpredictable with regard to such, as although the specification teaches that GSTP1 mRNA was absent in Hep3B cells, Imai et al (Carcinogenesis, vol. 18, pp 545-551, 1997) teach that expression of GST-P mRNA (rat π form of GST) was high in all rat hepatocellular carcinoma samples tested (see abstract, lines 16-18, Fig. 4). Further, Steinmetz et al (Carcinogenesis, vol.

19, pp 1487-1494, 1998) teaches that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms (see abstract, lines 16-20). Therefore, based on the lack of guidance in the specification as to an association between a hepatic cell proliferative disorder and hypermethylation of CpG in any GST nucleic acid or a decrease in GST mRNA expression in any mammal, for example, the skilled artisan would have to screen a large number of organisms to determine wether a hepatic cell proliferative disorder can be detected in any organism by detecting hypermethylation of CpG in a GST nucleic acid or a decrease in mRNA expression. The result of such analysis is unpredictable given that the art teaches that GST-P mRNA was high in all rat hepatocellular carcinoma samples tested and that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms. Therefore, the skilled artisan would be required to perform undue experimentation to practice the invention as broadly as it claimed.

With regard to detecting a hepatic cell proliferative disorder by detecting a decreased level of GST RNA in comparison to GST RNA levels in normal cells, although the specification teaches that GSTP1 mRNA was absent in Hep3B cells, the specification also teaches that normal hepatocytes generally fail to express GSTP1. Further, the specification does not teach comparing the level of GST mRNA expression in HCC cases versus normal hepatocytes, therefore, based on

the lack of guidance from the specification, and the fact that the specification teaches that normal hepatocytes generally fail to express GSTP1, it is unpredictable as to whether a hepatic cell proliferative disorder, or hepatocellular carcinoma, can be detected by merely detecting a decrease in GSTP1 mRNA expression. The art is further unpredictable with regard to GST expression and hepatocarcinogenesis. De Oliveira et al (Arquivos de Gastroenterolgia, 1990, vol. 27, pp 83-94, English abstract provided) teaches that serum levels of glutathione S transferase was increased in 64% of patients with hepatocellular carcinoma (lines 1-3 of abstract). Therefore, based on the lack of guidance from the specification, and the unpredictability taught in the art with regard to expression of GST in hepatocellular carcinoma, the skilled artisan would have to first determine whether a change in mRNA expression of any GST form (four are taught in the specification, at page 7, line 11: π, μ, α, and θ) exists between hepatocytes from tumor tissue and hepatocytes from normal tissue, to determine if a lack of GSTP1 or [any GST] expression is indicative of a hepatic cell proliferative disorder. Given that the specification teaches that normal hepatocytes generally fail to express GSTP1, such analysis would require trial and error, the outcome of which is unpredictable, thus constituting undue experimentation.

With regard to detecting methylation or hypermethylation in any region of a GST nucleic acid, the specification does not teach an association between HCC and hypermethylation in any region of the nucleic acid encoding GST, and instead teaches that somatic DNA hypermethylation changes present in HCC DNA consistently affected the gene promoter (p. 7,

lines 1-4). The specification also teaches that hypermethylation of the promoter region reduces the expression of GSTP1 in liver tissue (p. 8,line 22). Further, Steinmetz teaches, with regard to rat GST-P, that five of the eight CpG sites between -235 and +140 of the GSTP promoter regions, are located within consensus sequences for the DNA binding proteins Sp1 (whose binding is essential for GSTP transcription) and E2F, indicating at least one possible mechanism that could potentially lead to transcriptional activation of GSTP in hepatocellular foci and neoplasms during rat hepatocarcinogenesis (see abstract, last 3 sentences, and p. 1490). Steinmetz further teaches that methylation of critical cytosines within the promoter region, rather than all CpG associated cytosines, may be a determining factor in regulation of GSTP expression. Therefore, based on the teaching in the specification and the art, regulation of GSTP or GSTP1 can be regulated by hypermethylation of the promoter region. There is no teaching or guidance in the specification, however, that hypermethylation in an intron or exon, for example, of GSTP1 would lead to decreased expression of GSTP1 or be associated with hepatocellular carcinoma. Undue experimentation would be required of the skilled artisan to practice the invention as broadly as is claimed. The skilled artisan would have to screen a large number of hepatocellular carcinoma samples and normal hepatocytes, and using trial and error analysis, would have determine the level of methylation of CpG dinucleotides in all regions of the gene encoding GSTP1 to determine whether hypermethylation in any region of the gene is associated with HCC or any hepatic cell proliferative disorder. Such analysis would require trial and error, the outcome of which is unpredictable, thus constituting undue experimentation.

Response to Arguments

The response traverses the rejection. The response asserts (p. 8, para 2) that the examiner has provided no evidence to call into question the enablement of the claims. This argument has been thoroughly reviewed but was found unpersuasive in view of the rejection set forth in the previous office action and reiterated in the present office action (see section 5 above), which provides a detailed discussion, among other things, of both the teachings in the specification as well as conflicting teachings in the pertinent art, with regard to the instantly claimed invention.

The response further asserts (p. 8, para 3) that the examiner's assertion that only hepatocellular carcinoma can be detected according to the methods of the present invention is erroneous. The response also asserts that those skilled in the art recognize that a direct correlation exists between the presence of methylated CpG containing GST nucleic acid in a subject and hepatic cell proliferative disorders. The response cites the specification as teaching that human liver carcinogenesis proceeds via accumulation of "CpG island" hypermethylation of GST nucleic acid and therefore it is clear that *any* hepatic cell proliferative disorder can be detected by detecting a methylated CpG containing GST nucleic acid. This argument has been thoroughly reviewed but was found unpersuasive. Firstly, the citations in the specification, referenced in the response are either a reiteration of the claims ("summary of the invention", p. 4, lines 8-11) or with regard to HCC, not to *any* hepatocellular proliferative disorder such as disorders that include non cancerous tumors or growths such as cysts. Secondly, the response provides no examples that "the art recognizes that a direct correlation exists between the

presence of methylated CpG containing nucleic acid and hepatic cell proliferative disorders". In addition, as stated in the previous office action, the specification provides no guidance or working examples that any hepatic cell proliferative disorder can be detected by only detecting methylated CpG containing GST. Further, Herman et al (US Patent 6,017,704) teaches that human genes are known to be methylated (see col. 4, lines 1-5), including GST pi (human GST pi is GSTP1), and that hypermethylation of GST pi is associated with neoplastic tissue. Therefore, because the art teaches that GSTP1 inherently exists in a methylated state, the skilled artisan would not be able to detect a hepatic cell proliferative disorder based solely on the detection of methylated CpG containing GST nucleic acid.

The response traverses (pp8-9, bridging para) that the conclusion of the examiner, that those skilled in the art would not be able to detect a hepatocellular proliferative disorder based solely on detection of methylated CpG containing GST nucleic acid, is in error. The response asserts that those skilled in the art readily recognize that [the] invention methods detect nucleic acid which exhibits "aberrant methylation" of CpG rather than controlled methylation taught by Herman as is specified in claim 1. This argument has been thoroughly reviewed but was found unpersuasive because claim 1 is not drawn to detecting hypermethylation or "aberrant methylation", but instead recites "detecting a methylated CpG containing...". Because Herman teaches that GST pi is inherently methylated, the claim does not make clear that the method is drawn to hypermethylation or "aberrant methylation" as stated in the response.

The response further traverses (p. 9, para 2) the examiner's assertion that the specification provides no guidance with regard to detecting CpG containing GST nucleic acid in any biological fluid and that those skilled in the art recognize that inappropriately proliferating cells are invasive. This argument has been thoroughly reviewed but was not found persuasive. While it is known that inappropriately proliferating cells are invasive (ie: metastasis), the specification has provided no guidance that enough of any type of inappropriately proliferating hepatic cells could be detected in any biological fluid, such as ejaculate or urine. The response further asserts that the specification sets forth that the nucleic acid containing specimen used for detection of methylated CpG may be from any source, including for example colon, blood and lymphatic tissue. This argument has been thoroughly reviewed but was found unpersuasive. Firstly, the specification provides no evidence that any *hepatic* cell proliferative disorder can be detected from a nucleic acid containing specimen from colon tissue. As stated in the previous office action, hypermethylation of GST pi is associated with neoplastic tissue (p 9, line 6), however the specification has not taught whether detection of hypermethylated DNA encoding GST pi in ejaculate, for example, would be indicative of HCC or prostate cancer (it is known in the art that hypermethylation in the promoter region of GSTP1 is associated with prostate cancer, see Nelson et al., US Patent 5,552,277, example 5).

The response traverses (pp 9-10, bridging para) the examiner's position that the specification has not established an association between a hepatic cell proliferative disorder and detection of methylated CpG containing GST nucleic acid in *any* subject. The response asserts

that level of GST-P mRNA present in a specimen has no bearing on the detection of methylated CpG containing nucleic acid. This argument has been thoroughly reviewed but was found unpersuasive. Firstly, it is noted that the claims (specifically 16, 28 and 40) encompass detecting a hepatic cell proliferative disorder by detecting a decreased level of GST RNA in any subject (claims 28 and 40), while Imai et al (Carcinogenesis, vol. 18, pp 545-551, 1997) teach that expression of GST-P mRNA (rat π form of GST) was high in all rat hepatocellular carcinoma samples tested (see abstract, lines 16-18, Fig. 4). Therefore, the rejection set forth that although the specification teaches that GSTP1 mRNA was absent in human Hep3B cells, the art teaches conflicting results with regard to correlating mRNA expression in a rat based on expression in human tissue as Imai teaches that expression of GST-P mRNA was high in all rat hepatocellular carcinoma samples tested. With regard to the limitation in the claims with respect to detection of methylation of CpG containing GST nucleic acid, the rejection set forth that Steinmetz teaches that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms (see abstract, lines 16-20). Therefore, based on the lack of guidance in the specification as to an association between a hepatic cell proliferative disorder and hypermethylation of CpG in any GST nucleic acid in any subject, the skilled artisan would have to screen a large number of organisms to determine wether a hepatic cell proliferative disorder can be detected in any organism by detecting hypermethylation of CpG in a GST nucleic acid. The result of such analysis is unpredictable given that the art teaches that

that while eight cytosines between -235 and +140 in the GST-P promoter region were methylated in a site specific manner in GSTP-negative control rat liver, these same sites were hypomethylated in four chemically induced GSTP-positive neoplasms.

The response traverses (p. 10, para 2) the examiner's assertion that the specification has not established that a hepatic cell proliferative disorder can be detected by detecting a decreased level of GST RNA in comparison to GST in normal cells. The response further states that the specification teaches that cancerous human liver cells fail to express GST RNA and that since detecting and comparing levels of GST RNA in cells merely requires routine analysis, undue experimentation is not required. This argument has been thoroughly reviewed but was found unpersuasive. As stated in the rejection, although the specification teaches that GSTP1 mRNA was absent in Hep3B cells, the specification also teaches that normal hepatocytes generally fail to express GSTP1. Therefore, it is unclear as to whether a decrease in GSTP1 mRNA is indicative of a hepatic cell proliferative disorder. The rejection further stated that the specification does not teach comparing the level of GST mRNA expression in HCC cases versus normal hepatocytes, therefore, based on the lack of guidance from the specification, and the fact that the specification teaches that normal hepatocytes generally fail to express GSTP1, it is unpredictable as to whether a hepatic cell proliferative disorder, or hepatocellular carcinoma, can be detected by merely detecting a decrease in GSTP1 mRNA expression. The rejection also made note of the fact that the art is further unpredictable with regard to GST expression and hepatocarcinogenesis. De Oliveira et al (Arquivos de Gastroenterolgia, 1990, vol. 27, pp 83-94, English abstract provided)

teaches that serum levels of glutathione S transferase was increased in 64% of patients with hepatocellular carcinoma (lines 1-3 of abstract). Therefore, based on the lack of guidance from the specification, and the unpredictability taught in the art with regard to expression of GST in hepatocellular carcinoma, the skilled artisan would have to first determine whether a change in mRNA expression of any GST form (four are taught in the specification, at page 7, line 11: π, μ, α, and θ) exists between hepatocytes from tumor tissue and hepatocytes from normal tissue, to determine if a lack of GSTP1 or [any GST] expression is indicative of a hepatic cell proliferative disorder. Given that the specification teaches that normal hepatocytes generally fail to express GSTP1, such analysis would require trial and error, the outcome of which is unpredictable, thus constituting undue experimentation.

The response further traverses (p. 10, last para) the examiner's position that the specification does not teach an association between hepatocellular carcinoma and hypermethylation in any region of the nucleic acid encoding GST. The response asserts that although the example in the specification is illustrated with reference to CpG island hypermethylation in the promoter region of nucleic acid, the examiner has provided no evidence to call into question applicants claims that CpG island hypermethylation in any region of a nucleic acid encoding GST positively correlates with hepatocarcinogenesis. This argument has been thoroughly reviewed but was found unpersuasive. In the rejection the examiner cited the teachings of Steinmetz with regard to teachings of predictability and unpredictability in the pertinent art. The rejection stated that Steinmetz teaches [see p 12 of previous office action as

well as section 5 above], with regard to rat GST-P, that five of the eight CpG sites between -235 and +140 of the GSTP promoter regions, are located within consensus sequences for the DNA binding proteins Sp1 (whose binding is essential for GSTP transcription) and E2F, indicating at least one possible mechanism that could potentially lead to transcriptional activation of GSTP in hepatocellular foci and neoplasms during rat hepatocarcinogenesis (see abstract, last 3 sentences, and p. 1490). Steinmetz further teaches that methylation of critical cytosines within the promoter region, rather than all CpG associated cytosines, may be a determining factor in regulation of GSTP expression. The rejection then concludes that based on the teaching in the specification and the art, regulation of GSTP or GSTP1 can be regulated by hypermethylation of the promoter region but that there is no teaching or guidance in the specification that hypermethylation in an intron or exon, for example, of GSTP1 would lead to decreased expression of GSTP1 or be associated with hepatocellular carcinoma. The response's assertion that those skilled in the art are only required to perform routine analysis to determine the level of CpG island hypermethylation in any region of the polynucleotide is not found persuasive as the art suggests that it is hypermethylation *in the promoter region* that affects GSTP expression rather than all CpG associated cytosines. Because the specification has not provided any evidence to the contrary, the specification fails to provide a predictable correlation between hypermethylation in any region of the nucleic acid encoding GST and either altered GST expression or hepatocellular carcinoma or any hepatocellular proliferative disorder. Therefore, the rejection is maintained.

Conclusion

6. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

7. No claims are allowable.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jehanne Souaya whose telephone number is (703)308-6565. The examiner can normally be reached Monday-Friday from 9:00 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jehanne Souaya

Jehanne Souaya
Patent examiner
Art Unit 1634
1/7/03